The bovine herpesvirus 1 gene encoding infected cell protein 0 (bICP0) can inhibit interferon-dependent transcription in the absence of other viral genes

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The infected cell protein 0 (bICP0) encoded by Bovine herpesvirus 1 (BHV-1) stimulates viral gene expression and productive infection. As bICP0 is expressed constitutively during productive infection, it is considered to be the major viral regulatory protein. Like other alphaherpesvirus ICP0 homologues, bICP0 contains a zinc RING finger near its N terminus that activates transcription and regulates subcellular localization. In this study, evidence is provided that bICP0 represses the human beta interferon (IFN-β) promoter and a simple promoter with consensus IFN-stimulated response elements following stimulation with double-stranded RNA (polyinosinic–polycytidylic acid), IFN regulatory factor 3 (IRF3) or IRF7. bICP0 also inhibits the ability of two protein kinases (TBK1 and IKKε) to activate IFN-β promoter activity. The zinc RING finger is necessary for inhibiting IFN-dependent transcription in certain cell types. Collectively, these studies suggest that bICP0 activates productive infection by stimulating viral gene expression and inhibiting IFN-dependent transcription.

Infection of cattle with Bovine herpesvirus 1 (BHV-1) leads to conjunctivitis, pneumonia, genital disorders, abortions and ‘shipping fever’, an upper-respiratory infection (Tikoo et al., 1995). Infection of bovine cells causes rapid cell death and an increase in apoptosis (Devireddy & Jones, 1999). Viral gene expression during productive infection is regulated temporally in three phases: immediate-early (IE), early (E) and late (L) (Jones, 2003).

The bICP0 protein activates viral gene expression and is encoded by IE transcription unit 1 (IEtu1) (Wirth et al., 1992; Everett, 2000). bICP0 RNA is expressed constitutively during productive infection because the gene has an IE and E promoter (Fraefel et al., 1994). bICP0 (Fig. 1a) and herpes simplex virus type 1 (HSV-1) ICP0 proteins contain a well-conserved C3HC4 zinc RING finger, near their respective N termini, that is crucial for transcriptional activation (Everett, 1987, 1988; Everett et al., 1993; Inman et al., 2001). ICP0 (Maul et al., 1993; Maul & Everett, 1994; Everett et al., 1997, 1999a, b) and bICP0 (Parkinson & Everett, 2000; Inman et al., 2001) colocalize with and disrupt the promyelocytic leukaemia protein-containing nuclear domains (ND10 or PODS).

bICP0 associates with histone deacetylase 1 (HDAC1) and can relieve HDAC1-mediated transcriptional repression (Zhang & Jones, 2001). When BHV-1 DNA is transfected into permissive cells, plaque formation is inefficient unless bICP0, HSV-1 ICP0, the adenovirus E1A gene or E2F4 is included in the transfection mix (Inman et al., 2001; Geiser & Jones, 2003). The E1A protein (Chakravarti et al., 1999; Hamamori et al., 1999), E2F4 (Attwooll et al., 2004) and bICP0 (Zhang & Jones, 2001) interact with HDAC1-containing complexes, suggesting that sequestering HDAC1 stimulates BHV-1 productive infection.

HSV-1 infection of cultured human cells induces interferon (IFN) production. ICP0, ICP34-5 and Us11 are the known viral genes that inhibit IFN activation after infection (Mossman et al., 2000, 2001; Mossman & Smiley, 2002; Peters et al., 2002; Lin et al., 2004). The viral glycoprotein gD induces IFN-α production in mononuclear cells, leading to IFN response factor 3 (IRF3) activation (Katze et al., 2002). Mice lacking type I and type II IFN receptors in combination with RAG-2 gene deletions die within a few days of BHV-1 infection, whereas infection of wild-type (wt) mice does not lead to clinical symptoms (Abril et al., 2004). To date, the BHV-1 genes that regulate IFN have not been identified.

To test whether bICP0 inhibited IFN-dependent transcription in the absence of other viral genes, transient-transfection assays were performed using a wt bICP0...
Fig. 1. Regulation of IFN-β promoter activity by bICP0. (a) bICP0 contains two transcriptional-activation domains (TAD), a zinc RING finger (Wirth et al., 1992), an acidic domain and a nuclear-localization signal (NLS) (Zhang et al., 2005). The Sal site was used to construct the ΔbICP0 construct. Two amino acids were mutated in the bICP0 zinc RING finger: aa 13 was changed from C to G and aa 51 from C to A (13G/51A) (Inman et al., 2001). The bICP0 constructs contain the SV40 poly(A) addition site at the 3′ terminus of the bICP0 insert. Numbers denote amino acid positions. (b) Approximately 1 × 10^5 neuro-2A cells in a 60 mm dish were transfected with 1 μg IFN-β promoter (Zhang et al., 2005). Certain cultures were also cotransfected with 1, 2 or 3 μg of the wt bICP0 construct or a plasmid containing the BHV-1 LR gene. All cultures were transfected with 50 μg poly(IC) ml⁻¹. (c) Neuro-2A cells (approx. 1 × 10^5 cells in a 60 mm dish) were transfected with 1 μg IFN-β promoter. Some cultures were cotransfected with 1, 2 or 3 μg wt bICP0 construct and 2 μg IRF3 or IRF7, which yielded maximal stimulation of IFN-β promoter activity. Increasing concentrations of wt bICP0 constructs were also transfected with 1 μg LR promoter CAT construct (p0.95cat/1) (Jones et al., 1990). Plasmid DNA was maintained at the same concentration by including a blank expression vector (pcDNA3.1). At 40 h after transfection, cell lysate was prepared by three freeze–thaw cycles in 0.25 M Tris (pH 8.0). CAT activity was measured in the presence of 0.2 μCi (7.4 kBq) [14C]chloramphenicol and 0.5 mM acetyl-coenzyme A (Inman et al., 2001; Zhang & Jones, 2001). The amount of acetylated chloramphenicol was measured with a Bio-Rad Molecular Imager FX following separation by thin-layer chromatography. Basal values for the IFN-β promoter were normalized to 1 and other values are presented as fold activation relative to basal promoter levels. Results are means of at least three experiments.
construct, a zinc RING finger mutant (13G/51A) or a deletion mutant that lacks the last 320 aa of bICP0 (ΔbICP0) (Fig. 1a). These constructs express similar levels of bICP0 in transiently transfected cells (Zhang & Jones, 2001; Henderson et al., 2004; Zhang et al., 2005). We predicted that bICP0 regulates IFN-β-dependent transcription because HSV-1 ICP0 inhibits IFN-dependent transcription (Mossman et al., 2000, 2001; Mossman & Smiley, 2002; Lin et al., 2004). Except for the zinc RING finger located near the N terminus of bICP0 (Fig. 1b), there is little similarity between bICP0 and ICP0, making it necessary to test formally whether bICP0 inhibits IFN-dependent transcription.

The IFN-β promoter was initially tested because it is activated strongly by early events that occur following virus infection (Katze et al., 2002). For these studies, a plasmid containing the human IFN-β promoter (−110 to +20) linked to the bacterial chloramphenicol acetyltransferase (CAT) gene was used. This promoter construct was obtained from Dr Stavros Lomvardas (Columbia University, NY, USA) and it contains elements that are activated by virus infection (Munshi et al., 2001). As reported previously (Peng et al., 2005), IFN-β promoter activity was low in mouse neuroblastoma cells (neuro-2A cells) (Fig. 1b). Double-stranded RNA [polynosinic–polycytidylic acid, poly(IC)] increased IFN-β promoter activity by more than 11-fold in neuro-2A cells (Fig. 1b). bICP0 decreased IFN-β promoter activity in a dose-dependent manner and at the highest concentration of bICP0 tested, IFN-β promoter activity was reduced by approximately 10-fold. We also tested a plasmid that expresses the latency-related (LR) gene products because the LR gene overlaps bICP0 (Jones, 2003). LR gene products were unable to inhibit IFN-β promoter activity in transiently transfected neuro-2A cells (Fig. 1b).

IRF3 and IRF7 are transcription factors that are activated by phosphorylation following IFN induction (Barnes et al., 2002). Overexpression of IRF3 or IRF7 consistently stimulated IFN-β promoter activity by more than five- or sevenfold, respectively, in neuro-2A cells (Fig. 1c). With as little as 1 μg bICP0 added to the cotransfection mix, IFN-β promoter activity was reduced by more than twofold when activated by IRF3 and by fivefold when activated by IRF7 (Fig. 1c). When bICP0 was cotransfected with a BHV-1 LR promoter construct, bICP0 activated LR promoter activity by more than sevenfold, which confirmed the results of earlier studies (Bratanich & Jones, 1992). These studies indicated that bICP0 inhibited IFN-β promoter activity, but activated LR promoter activity in transiently transfected neuro-2A cells.

IRF3 is activated by phosphorylation in two steps (Barnes et al., 2002; Sarkar et al., 2004) and two protein kinases, IKKε and TBK1, coordinate IRF3 activation (Fitzgerald et al., 2003; Sharma et al., 2003). Cytomegalovirus expression plasmids containing these protein kinases were obtained from Dr Tom Maniatis (Harvard University, MA, USA). IKKε consistently activated IFN-β promoter activity in neuro-2A cells by more than sevenfold (Fig. 2a and Supplementary Table S1a, available in JGV Online). wt bICP0 or the ΔbICP0 mutant reduced IFN-β promoter activity by approximately 50 % using 1 μg bICP0, and repression occurred in a dose-dependent fashion. At the higher concentration of bICP0 (wt or ΔbICP0), activation of IFN-β promoter activity by IKKε was negated. In contrast, the 13G/51A zinc RING finger mutant was unable to inhibit IFN-β promoter activity to basal levels. Similar results were obtained in human 293 cells (data not shown).

Although TBK1 did not stimulate IFN-β promoter activity in neuro-2A cells, TBK1 stimulated IFN-β promoter activity by 2-7-fold in human epithelial cells (293) (Fig. 2b and Supplementary Table S1b). Induction of IFN-β promoter activity by TBK1 was repressed to basal levels by wt bICP0 or ΔbICP0, but not by the 13G/51A construct.

Activated IRF3 induces expression of IFN-α4 in mice or IFN-α1 in humans, and IRF3 cooperates with other transcription factors to activate the IFN-β promoter (Fitzgerald et al., 2003). IRF7 induced expression of IFN-β in cultured human cells (Bratanich et al., 1994). These results indicated that bICP0 inhibited IFN-β promoter activity, but activated LR promoter activity in transiently transfected neuro-2A cells.

**Fig. 2.** Regulation of the IFN-β promoter by IKKε and TBK1. (a) Neuro-2A cells (approx. 1 x 10^5 cells in a 60 mm dish) were transfected with 1 μg IFN-β CAT construct, 2 μg IKKε, and the designated bICP0 plasmids (1, 2 or 3 μg). (b) Approximately 1 x 10^5 293 cells in a 60 mm dish were transfected with 1 μg IFN-β CAT construct, 2 μg TBK1 and the designated bICP0 plasmids (1, 2 or 3 μg). CAT activity was measured as described in the legend to Fig. 1. The amounts of IKKε and TBK1 used for this study were optimal for activating the IFN-β promoter. Results are means of four independent experiments.
et al., 2003; Sharma et al., 2003; Sarkar et al., 2004). Transcription of other IFN-α subtypes requires IRF7, which is crucial for type I IFN-dependent immune responses in mice (Honda et al., 2005). IFN-stimulated response elements (ISREs) are present in many genes activated by IFN and are necessary for IFN induction. Consequently, we tested whether bICP0 inhibited ISRE-dependent transcription.

We subsequently tested whether bICP0 inhibited a minimal human immunodeficiency virus promoter construct with four consensus ISREs (pISRE). The ISRE elements in pISRE are from the ISG15 gene, and pISRE was obtained from Dr L. Zhang (University of Nebraska, NE, USA). IRF3 stimulated pISRE promoter activity by more than 50-fold in bovine cells (9.1.3), 40-fold in 293 cells or 15-fold in neuro-2A cells (Fig. 3a and Supplementary Table S2a). Activation of pISRE promoter activity by IRF3 was inhibited by almost threefold in 9.1.3 or neuro-2A cells and sixfold in 293 cells when 1 μg wt bICP0 was used in the transfection. The zinc RING finger mutant (13G/51A) was unable to effectively repress IRF3 induction of pISRE promoter activity in 9.1.3 cells. In 293 or neuro-2A cells, the 13G/51A mutant reduced pISRE promoter activity almost as efficiently as wt bICP0. The C-terminal bICP0 mutant (ΔbICP0) and wt bICP0 inhibited IRF3 induction of pISRE promoter activity with similar efficiency, suggesting that aa 1–356 were sufficient for inhibiting pISRE promoter activity.

IRF7 stimulated pISRE promoter activity by more than 15-fold in 9.1.3 cells, 67-fold in 293 cells or fourfold in neuro-2A cells (Fig. 3b and Supplementary Table S2a). Activation of pISRE promoter activity by IRF7 was inhibited by almost threefold in 9.1.3 or neuro-2A cells and sixfold in 293 cells when 1 μg wt bICP0 was used in the transfection. The zinc RING finger mutant (13G/51A) was unable to effectively repress IRF7 induction of pISRE promoter activity in 9.1.3 cells. In 293 or neuro-2A cells, the 13G/51A mutant reduced pISRE promoter activity almost as efficiently as wt bICP0. The C-terminal bICP0 mutant (ΔbICP0) and wt bICP0 inhibited IRF7 induction of pISRE promoter activity with similar efficiency, suggesting that aa 1–356 were sufficient for inhibiting pISRE promoter activity.

![Figure 3](https://example.com/figure3.png)

**Fig. 3.** Regulation of ISRE-dependent transcription by bICP0. Neuro-2A, 293 or 9.1.3 cells (1 × 10^5 in a 60 mm dish) were transfected with 1 μg pISRE promoter. Cultures were also cotransfected with 2 μg IRF3 (a) or IRF7 (b) and 1, 2 or 3 μg of wt bICP0, 13G/51A mutant or the ΔbICP0 mutant. Plasmid DNA was maintained at the same concentration by including a blank expression vector (pcDNA3.1) in the transfection mix. (c) The LR promoter (p0.95cat/1) was cotransfected with 1 or 2 μg wt bICP0 plasmid in 293 cells (empty bars) or 9.1.3 cells (filled bars). CAT activity was measured as described in the legend to Fig. 1. Basal values for the LR promoter or pISRE were normalized to 1 and the values presented are fold activation relative to basal promoter levels. Results are means of four experiments.
In all cell types examined, bICP0 were necessary to inhibit pISRE promoter activity following activation with IRF7 (Fig. 3b and Supplementary Table S2b). At higher concentrations of the 13G/51A plasmid, pISRE promoter activity was repressed in 9.1.3 cells almost as efficiently as by wt bICP0. In 293 cells, 1 μg bICP0 or ΔbICP0 inhibited the ability of IRF7 to activate pISRE promoter activity by more than 10-fold. Conversely, the 13G/51A construct repressed IRF7 induction of the pISRE promoter by only three- to fourfold in 293 cells. In neuro-2A cells, bICP0 (wt, the 13G/51A mutant or the ΔbICP0 mutant) repressed promoter activity with similar efficiency. As expected, bICP0 activated the LR promoter in 293 or 9.1.3 cells (Fig. 3c and Supplementary Table S2c). These studies suggested that the zinc RING finger was necessary for inhibiting pISRE promoter activity in certain cell types.

Collectively, these studies indicated that bICP0 repressed the IFN-β promoter (an early IFN response) and ISRE-dependent transcription (a relatively late IFN response). Conversely, bICP0 activated the LR promoter in 293, 9.1.3 and neuro-2A cells. These studies do not preclude the possibility that bICP0 inhibits other cellular promoters, nor do they explain how bICP0 inhibited IFN-β- or ISRE-dependent promoter activity. We suggest that, in general, interactions between bICP0 and cellular transcription factors stimulate viral transcription, but repress certain cellular promoters.

The two point mutations within the bICP0 zinc RING finger prevent activation of the thymidine kinase (TK) promoter and productive infection in all cell types that have been examined (Inman et al., 2001). In contrast, the zinc RING finger was only required to inhibit IRF3 induction of pISRE promoter activity in bovine cells (9.1.3 and IKKε; induction of IFN-β promoter activity, suggesting that the zinc RING finger was necessary for inhibiting a specific IRF3-dependent step. HSV-1-encoded ICP0 also represses the antiviral effects of IFN (Mossman et al., 2000; Mossman & Smiley, 2002) by inhibiting IRF3 and IRF7 induction of IFN-stimulated genes, and the zinc RING finger is required for blocking activation (Lin et al., 2004).

In all cell types examined, the ΔbICP0 deletion mutant efficiently inhibited pISRE and IFN-β promoter activity, suggesting that aa 357–676 were not important. The C terminus of HSV-1 ICP0 also does not play a major role in blocking IFN-dependent transcription (Lin et al., 2004). The ΔbICP0 construct does not activate a simple HSV TK promoter (Inman et al., 2001; Zhang & Jones, 2001), suggesting that bICP0 sequences required for activating transcription are distinct from sequences that inhibit IFN-dependent transcription. A recent study has identified several functional domains within bICP0 that are necessary for activating a simple viral promoter (Zhang et al., 2005). It will be of interest to identify the bICP0 functional domains that inhibit IFN-dependent transcription and to compare them with sequences necessary for activating viral transcription.

Although we believe that bICP0 is an important viral gene that inhibits the IFN response, it is likely that other BHV-1 genes antagonize the IFN response following infection. Support for this hypothesis comes from studies demonstrating that HSV-1 genes encoding ICP0, 34-5 and Us11 inhibit the IFN response (Mossman et al., 2000, 2001; Katze et al., 2002; Mossman & Smiley, 2002). BHV-1 apparently does not encode a 34-5 homologue, suggesting that other BHV-1 genes with similar functions exist. Future studies will attempt to identify other viral genes that regulate the IFN response, and elucidate their role in pathogenesis.

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References


